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Review

In vitro and *in vivo* approaches for the measurement of oral bioavailability of lead (Pb) in contaminated soils: A review

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ABSTRACT

We reviewed the published evidence of lead (Pb) contamination of urban soils, soil Pb risk to children through hand-to-mouth activity, reduction of soil Pb bioavailability due to soil amendments, and methods to assess bioaccessibility which correlate with bioavailability of soil Pb. Feeding tests have shown that urban soils may have much lower Pb bioavailability than previously assumed. Hence bioavailability of soil Pb is the important measure for protection of public health, not total soil Pb. Chemical extraction tests (Pb bioaccessibility) have been developed which are well correlated with the results of bioavailability tests; application of these tests can save money and time compared with feeding tests. Recent findings have revealed that fractional bioaccessibility (bioaccessible compared to total) of Pb in urban soils is only 5–10% of total soil Pb, far lower than the 60% as bioavailable as food-Pb presumed by U.S.-EPA (30% absolute bioavailability used in IEUBK model).

1. Introduction

Concern about potential risks from Pb in urban and garden soils have been widely expressed because of the history of soil contamination from domestic use of Pb paints, deposition of automotive Pb emissions, and from mine waste and smelter contaminated areas developed for housing (Hunt et al., 1992; Levin et al., 2008; Mielke et al., 1983). Among direct exposure pathways for Pb in urban environments, inadvertent ingestion of soil is considered the major concern compared with dermal and respiratory pathways (Chaney et al., 1989; Davies et al., 1990; Johnson and Bretsch, 2002; Paustenbach, 2000; Thornton et al., 1990; Thornton et al., 1995). In modeling risks from diet, water and soil Pb, U.S.-EPA presumes that soil-Pb is 60% as bioavailable as other dietary Pb (U.S.-EPA, 1994, 1999). The default value for soil Pb bioavailability is 30% (60% as bioavailable as Pb from water and food) in the Integrated Exposure Uptake Biokinetic (IEUBK) model (U.S.-EPA, 1994). Implications of soil Pb risk and several recommendations have been based on this assumed soil Pb fractional bioavailability figure (U.S.-EPA, 2001).

Inner city soils are considerably more contaminated than suburban soils, although exterior Pb paint scrapped to soil can cause high soil Pb contamination wherever it occurs, easily causing soil to exceed 10,000 mg Pb kg^{-1} (Murray and Hendershot, 2000). High soil Pb has become a worrisome source of risk to children because Pb has become widely dispersed in urban soils (Mielke et al., 1983, 1984, 2007; Demetriades et al., 2010; Laidlaw and Taylor, 2010). Soil Pb is a greater risk thru soil ingestion than thru uptake by garden food crops (Chaney et al., 1984; Chaney and Ryan, 1994). Thornton et al. (1985) concluded that "the concentration of lead in house dusts is significantly related to that in garden soil, and is highest at older homes." [In the UK and some other nations, "garden" refers to all land surrounding housing, while in the US, "garden" usually refers to soils used in food and flower production.] A meta-analysis of the contribution of soil vs. housedust to blood-Pb of urban children has shown that housedust is considerably more important and interior paint Pb comprised far greater risk than soil Pb (Lanphear et al., 1998). Analysis of housedust suggested that paint, road dust, and garden soil may all be important lead sources (Hunt et al., 1992). These contributions are a function of particle size, and the importance of the contributions is dependent on whether the apportionment is based on particle population or estimated particle volume or mass (Hunt et al., 1992).

Nriagu (1972, 1974) provided the first indication that the extremely insoluble Pb mineral pyromorphite $[Pb_5(PO_4)_3\,X$, where X is OH, Cl, or F] should be formed in Pb contaminated soils having some amounts of phosphorus compounds. Discovery of pyromorphite in

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urban soil was first reported when researchers in the UK found no correlation between high Pb levels in garden soil and housedust with elevated blood levels of children who lived with the soils, and scanning electron microscopy coupled with X-ray analysis of the soil grains revealed that many Pb-rich particles were composed of pyromorphite, a stable Pb mineral with a characteristic of low bioavailable Pb (Cotter-Howells and Thornton, 1991). Following this Ma et al. (1993) examined formation of chloro-pyromorphite from Pb compounds and soil Pb; their work suggested that this could be an effective soil Pb remediation technology. Later, pyromorphite was identified in some mine-waste and industrially contaminated soils (Cotter-Howells et al., 1994). Then Thornton et al. (1995) noted that "although lead of mine-waste origin may be present at elevated levels in dusts and soils, it does not necessarily contribute to elevated blood lead levels when the lead is present in relatively insoluble forms." Analysis of housedusts from hand-wipe samples collected from children in Winster, UK showed that in total, for the five soils, 28.4% of soil particles on hands fell into the "pyromorphite" class (Watt et al., 1993). Within the next few years Cotter-Howells (1996) and Cotter-Howells et al. (1999) discovered formation of this highly insoluble mineral within the rhizosphere of *Agrostis capillaris* roots, indicating that root growth could promote formation of pyromorphite, perhaps by the phosphate solubilizing activity of roots. Thornton et al. (2008) in another study, employing sequential chemical extraction, discovered that "significant proportions of Pb in brownfield contaminated soils were relatively insoluble and suggested that the low solubility of Pb did not necessarily present a risk to exposed population groups." However, bioavailability or bioaccessibility of soil/dust lead directly ingested by young children was not determined by these authors. Further, the common sequential extraction methods applied to contaminated soils actually promote transformation of the Pb species present (Scheckel and Ryan, 2004); only use of an *in situ* spectroscopic method such as X-ray absorption spectroscopy can measure the Pb species present in a contaminated soil material.

At present, for total Pb concentration, U.S. EPA and U.S. Department of Housing and Urban Development (HUD) have established a 400 ppm standard for children's play areas and an average of 1200 ppm standard in the remainder of the yard (U.S.-EPA, 2001). The Agency believes that more than 12 million homes exceed a 400 ppm yard-wide standard while at 1200 ppm Pb threshold, only 4.7 million homes exceed the standard.

Lead bioavailability in soils is largely controlled by phosphate, iron oxides, organic matter and pH. Iron (oxy)hydroxides [such as goethite and amorphous $Fe(OH)_3$ (ferrihydrite)] and organic matter create surface sorption or chelation sites for binding Pb^{2+}, and dissolved phosphate causes its precipitation (Appel and Ma, 2002; Brown and Chaney, 2003; Ryan et al., 2004; Traina and Laperche, 1999). In a similar way, an increase in pH also decreases Pb mobility and bioavailability as fewer H^+ ions are available to compete with Pb^{2+} ions for binding sites (Cao et al., 2008; Hettiarachchi and Pierzynski, 2004) in addition to the formation of Pb carbonate minerals.

For over a decade, researchers have explored *in situ* soil treatments such as phosphate (e.g., single/triple superphosphate, phosphoric acid, rock phosphate, monoammonium phosphate, bone meal), and amorphous iron oxides to reduce the phyto- and bioavailability of soil Pb (Basta et al., 2001; Porter et al., 2004; Brown et al., 2004; Brown et al., 1999; Cao et al., 2008; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001; Hettiarachchi and Pierzynski, 2002; Hodson et al., 2001, 2000; Laperche et al., 1996, 1997; McGowen et al., 2001; Moseley et al., 2008; Ryan et al., 2004; Sneddon et al., 2006, 2008; Sterrett et al., 1996; Yang et al., 2001). As noted above, while urban soils often have evidence of pyromorphite formation (Cotter-Howells, 1996), Pb mine wastes usually contain very low phosphate levels inhibiting natural formation of pyromorphite (Harwood et al., 1987).

Use of conventional soil extraction methods [5 mM diethylenetriaminepentaacetic acid (DTPA), 0.01 M $Ca(NO_3)_2$] to assess phytoavailable Pb was unable to identify the significant reduction in soil Pb bioavailability induced by application of phosphate or Fe-rich biosolids which were demonstrated through *in vivo* and *in vitro* trials (Brown et al., 2003; Ryan et al., 2004). Because conventional soil tests yield results unrelated to bioavailability of soil Pb risk to children, alternative tests are needed. In a recent study on soils contaminated with Pb and As, bioaccessibility of As and Pb measured in artificial gastric and small intestinal solutions decreased with phosphate and iron application except for the bioaccessibility of As in the gastric phase with single super phosphate addition (Cui et al., 2010). Combined application of phosphates and iron could be an effective approach to lower bioaccessibility of As and Pb, but had opposing effects on mobility of As and Pb in contaminated soils, the authors concluded.

Trials using animals for measurements of soil Pb *bioavailability* are expensive (about $30,000 per test soil with swine) and time consuming (Casteel et al., 2006). Thus, several chemical test methods have been developed to measure soil Pb *bioaccessibility* to animals (*in vitro* approach) starting with Ruby et al. (1993). These kind of *in vitro* approaches need not necessarily represent all the physiological processes provided bioaccessible Pb is well correlated with bioavailable Pb (Ruby et al., 1993; Drexler and Brattin, 2007; Scheckel et al., 2009). In order to obtain improved bioaccessibility tests, numerous authors have conducted tests of variation of the methods by adding different digestion factors from the human digestive system assuming that the more lifelike the method, the better the reliability would be (e.g., Oomen, 2000; Oomen et al., 2002, 2003a,b, 2004, 2006). Others (e.g., Thums et al., 2008) have simply used 0.12 M HCl ignoring the pH buffering aspect of stomach secretions and ingested soil and foods. In our view, the relevant issue is the relationship of the bioaccessibility test and an acceptable bioavailability measure, where bioavailability to humans is the ultimate best estimate.

Ruby et al. (1993) introduced a chemical extraction method to estimate the bioavailability of soil Pb which was well correlated with bioavailability measured by rabbits, and this extraction result was labeled "*bioaccessibility*" to make clear it was not a biological measurement of bioavailability. Within the next few years, Ruby et al. (1996, 1999) extended the development of their earlier bioaccessibility extraction method and called it the Physiologically-Based Extraction Test (PBET). The modified test was more complicated, but seemed well correlated with results of available feeding studies. After this development, there were many studies along similar lines by several researchers (Oomen, 2000; Oomen et al., 2002, 2003a,b, 2004, 2006) who tried to see if some chemicals similar to gastric juice/GI secretions could simulate living animal digestion behavior for measurement of Pb (and other metals as well) entry to the systemic circulation (Intawongse and Dean, 2006; Hooda, 2010). Presently, a variety of *in vitro* GI methods have been developed and proposed, with multi-phase tests involving a wide range of pH (1.07–7.5), different size fractions of soil (<2.00 mm to <125 μm), residence time (5 s to 16 h), temperature 20–37 °C, and soil:solution ratio (1:2 to 1:5000) (Wragg and Cave, 2002; Intawongse and Dean, 2006). The pH values used in such tests don't necessarily represent a close approximation of the human GI system (Hooda, 2010). Mean stomach fasting pH ranges from 1 to 4 in children (Anderson et al., 1999), and from 1.5 to 2 in adults (Charman et al., 1997), but when food is consumed stomach pH commonly rises to 6 (Malagelada et al., 1979).

Recently Drexler and Brattin (2007) introduced a modified *in vitro* procedure for estimation of lead bioaccessibility that was well correlated with the results of bioavailability tests using pigs fed mine waste and smelter contaminated soils, and this method

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was adopted by U.S.-EPA (2007) for assessment of risk from mine waste and other Superfund site soils. Bioaccessibility extractions are an important tool as such tests can be conducted at a fraction of the cost of in vivo tests, allowing analysis of a sufficient number of samples to more fully characterize soil Pb risk variability at a site to support remediation decisions.

Pb-phosphates are significantly less soluble than galena (PbS), anglesite ($PbSO_4$), cerrusite ($PbCO_3$), litharge (PbO), and crocoite ($PbCrO_4$), Pb-solids commonly reported in contaminated soils (Ruby et al., 1994; Laperche et al., 1996). Nriagu (1972, 1974); Porter et al. (2004), Santillan-Medrano and Jurinak (1975), and Sauvé et al. (1997) suggested that control the solubility of Pb in noncalcareous soils might be controlled by Pb-phosphate phases. Keeping in view very low solubilities of lead-phosphate compounds and their discovery in some contaminated soils, a lot of efforts have been made to demonstrate the formation of similar compounds on lead contaminated soil with the introduction of phosphate in order to decrease bioavailability of this metal (Ryan et al., 2004).

Ryan et al. (2004) tested methods to reduce the bioavailability of Pb in Pb-smelter contaminated urban soils in a field test in Joplin, Missouri (MO), USA, where different field treatments to reduce Pb bioavailability were installed in 1997. In 2000, soil samples were collected from all plots to conduct feeding tests and chemical extractions to assess bioaccessibility. The most important finding from the Joplin study was that phosphate treatment could reduce soil Pb bioavailability to humans by as much as 69%. Another important finding was that the Drexler and Brattin (2007) method conducted at pH 1.5 seriously underestimated the ability of phosphate and other treatments to reduce soil Pb bioavailability to animals. In particular, the pH 1.5 extraction estimated that bioaccessibility had been reduced by only 18%, while feeding of phosphate-amended soils (1% H_3PO_4 treated and sampled after 18-months) to humans showed that bioavailability had been reduced by 69% (42.2% vs. 13.1% absolute bioavailability). Testing other initial pH levels with the 0.4 M glycine bioaccessibility extraction test of Drexler and Brattin (2007) showed that extraction at pH 2.2 or 2.5 gave reduction in bioaccessibility (64% at pH 2.5) which better matched the reduction in bioavailability in the feeding tests for humans, rats and swine. For the rat, reduction in soil lead bioavailability for the soil treated with 1% H_3PO_4 was 67% (21.7% vs. 7.2% absolute bioavailability 18 month sample). For swine, reduction in soil lead bioavailability for the soil treated with 1% H_3PO_4 was 29% for the 3-month sample, 38% (34.8% vs. 21.6% absolute bioavailability) for the 18-month sample and 71% for the 32-month sample (Ryan et al., 2004; Scheckel and Ryan, 2004; Brown et al., 2004).

It is increasingly recognized that the response of an at-risk population is not controlled by the total metal concentration, but instead is controlled by only the biologically available portion, which is dependent on the route of exposure, the pharmacokinetics of the organism, and the speciation of the contaminant. In urban garden soils, where Pb contamination is prevalent and gardeners lack financial resources to remove and replace soil, it is crucial to recognize the factors which might affect Pb bioavailability (Clark et al., 2006). Researchers have difficulty in agreeing on what soil Pb concentration in urban gardens poses risk. For example, some researchers advocate raised beds and compost application strategies in addition to soil pH adjustments as well as other long-term remediation or stabilization techniques for urban gardens (Clark et al., 2006; Farfel et al., 2005).

2. Need of an easier and low-cost bioaccessibility test method

In general, there are two common approaches for *in vitro* extractions to determine bioaccessible Pb and other metals or metalloids in soils. The physiologically based extraction tests (PBETs) based methodology employs components like simulated gastric solution at 37 °C, stomach and intestinal pH, soil-to-solution ratio, and mixing, where such parameters mimic conditions of the fasting human digestive tract (Hooda, 2010; Hettiarachchi and Pierzynski, 2004; Ruby, 2004; Wragg and Cave, 2002; Ruby et al., 1999). The approach of other bioaccessibility extraction methods is not necessarily to mimic a human's GI tract, but to solubilize trace elements using "simple" solutions that yield a high correlation with the bioavailable elements in the soil. This approach uses the measured bioaccessibility to estimate bioavailability using mathematical relationships gleaned from *in vivo* studies. In theory, if a mathematical relationship exists between *in vitro* and *in vivo* trials results, then the fluid composition of the *in vitro* test does not necessarily need to be a close mimic of a GI tract (Smith et al., 2010). The Relative Bioaccessibility Leaching Procedure (RBALP) (0.4 M glycine·HCl adjusted to pH 1.5) (Drexler and Brattin, 2007) uses simple acidic solution to solubilize contaminants from soil. Both the *in vitro* approaches do not take in to account absorption, metabolism, excretion, or sequestration of contaminants.

Several *in vivo* studies employing laboratory animals suggest that the oral bioavailability of contaminants in soil could be significantly lower than that measured for contaminants in matrices such as food and liquids (Chaney et al., 1989; Freeman et al., 1992, 1994, 1996; Dieter et al., 1993). In evaluating the relative bioavailability of Pb from Butte, Montana, mine waste soils in rats, Freeman et al. (1992) found that "the overall relative bioavailability values were 20% based on blood data, 9% based on bone data, and 8% based on liver data." It is important to note that the dose–response for Pb-acetate in these tests was linear, while the tissue Pb response to soils plateaued with increasing dose. This is believed to result because with an increased amount of soil sorption surfaces in the stomach and intestine at the high soil dose rate, Pb solubility and bioavailability are lower. In a second study, Freeman et al. (1994) reported that absolute bioavailability of mining waste lead in soil (administered in purified AIN diets) was about 3% based on blood data and less than 1% based on bone and liver analyses. Davis et al. (1992) employed *in-vivo* (rat as test animal) and *in-vitro* approaches to investigate Pb (and As) risk from mine waste impacted soils (Butte, Montana, previously used by Freeman et al., 1992) and found that when test animals were fed with the contaminated soils, about 6% of total Pb was solubilized in the small intestine, signifying that in-vitro approach might be quite useful for estimating bioavailable Pb. Strong correlations were found between administered and tissue lead concentrations when rats were fed with four lead compounds varying in Pb solubility (Dieter et al., 1993). Maximum blood lead concentrations were attained with administration of highly soluble Pb sources lead acetate and lead oxide (compared to less soluble sources lead sulfide and lead ore) both in blood and bones. Schoof et al. (1995) in an experiment using Sprague–Dawley rats fed with Pb contaminated soil (abandoned smelter site) reported that bioavailability of soil lead was 41% relative to lead acetate, and that tissue Pb response to increasing dietary soil approached a plateau. The data suggested that soil Pb bioavailability at that location was 20% based on the assumption that from diet, children absorb 50% Pb. The bioavailability figure is more than double, as per findings of Freeman et al. (1992) who used soil from same location. To test the theory whether soil in stomach might be a sink itself for Pb, Freeman et al. (1996) in rat trials tested Pb acetate treatments alone compared with co-administration of control soil, which significantly reduced Pb absorption; this was similar to Chaney et al. (1989) who found about 50% reduction in Pb bioavailability to rats when a control soil was added to the diet at 5%. Such *in vivo* feeding studies with rats demonstrate that soil can have lower Pb

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bioavailability suggesting that the 30% absolute bioavailability of Pb presumed by EPA may not be correct for all soils.

In general bioavailability of ingested soluble lead in adults has been found to vary from 2–10% when ingested with a meal to 60–80% when ingested after a fast (e.g., James et al., 1985). And for young children, balance studies indicate that average daily absorption of Pb is greater as compared to adults (Alexander et al., 1974; Ziegler et al., 1978). In human adult trials, dietary Ca (and to some extent phosphate) has also been reported to inhibit Pb absorption considerably (e.g., Blake and Mann, 1983). Possible mechanism for this interaction might be complexation of the metal with Ca (and phosphate) in the GI tract and competition for a common transport protein (Barton et al., 1978; Heard and Chamberlain, 1982). Heard and Chamberlain (1983) also reported that "fasting humans absorbed 40–50% of ^{203}Pb taken in distilled water, irrespective of the addition of Pb carrier up to 100 μg per dose. When taken with tea or coffee, uptake averaged 14% and with beer 19%. Much lower uptakes, ranging from 3 to 7%, were found when ^{203}Pb was taken with a meal, or incorporated in the form of liver + kidney (Heard and Chamberlain, 1983) or spinach (Heard et al., 1983) which were eaten as part of a meal, or when taken with large amounts of calcium or phosphate". Later James et al. (1985) administered ^{203}Pb as Pb acetate to human adults on the 12th hour of a 19 h fast and retention measured a week later depicted a figure of 61% on whole body count basis. Consuming a balanced meal with added soluble ^{203}Pb reduced Pb uptake to 4% and influence of the food lasted for up to 3 h after consuming a meal. A general consensus is that constituents of food in the GI tract decrease ingested lead absorption, although the exact mechanisms by which this occurs are not entirely understood.

Maddaloni et al. (1998) conducted a human feeding trial for Pb-rich soil from the Kellogg, ID, Superfund site employing Pb stable isotopes (^{206}Pb/^{207}Pb ratio) to measure Pb absorption by the test subjects (used subjects whose blood Pb isotope ratio was different from that of the test soil to allow a valid assay of absorbed Pb). They found that on average, 26.2% of the administered soil Pb dose was absorbed when fed after overnight fastening. However, adults who ingested soil immediately after a standardized breakfast absorbed only 2.5% of the soil Pb dose. These findings are in good agreement with previously reported results of James et al. (1985). The Maddaloni team also fed Joplin, MO soil to fasting humans and found 42.2% absolute bioavailability of Pb for the untreated soil but only 13.2% for the soil treated with 1% P (phosphoric acid treatment), i.e., 69% reduction in bioavailability for treated soil (Ryan et al., 2004).

Before introduction of the first *in vitro* method for soil Pb bioaccessibility (Ruby et al., 1993), bioavailability of trace elements and minerals was assessed through inexpensive but simple and rapid techniques as an alternative to animals and human in vivo studies (Shen et al., 1994). Ruby et al. (1993), following a modified version of the method of Miller et al. (1981) to estimate food Fe bioavailability, evaluated the *in vitro* bioaccessibility of ingested mine waste Pb using New Zealand white rabbits. This version of the *in vitro* method, modeled after methods to assay bioavailable iron in foods, used a soil to solution ratio of 1:10, stomach fluid with pepsin and organic acids, stomach phase adjusted at pH 1.3 by means of HCl, a dialysis bag containing $NaHCO_3$ to neutralize the stomach fluid gradually for the intestinal phase of the test, at 37 °C. When tested for four mine waste contaminated soils, the in vitro results were in close agreement with the bioavailability results (1–6% bioavailability). In this study, presence of low Pb soil gave a reduction of 8% in Pb bioaccessibility from Pb acetate in the stomach phase. Later, a modified version of previous *in vitro* test that is now widely known as PBET (physiologically based extraction test) was introduced (Ruby et al., 1996). The method although similar with that of its older version (Ruby et al., 1993), possesses many modifications like wider soil:solution ratio (1:100), adjustment in stomach pH to 2.5 with inclusion of small amounts of porcine bile/porcine pancreatin after removal of $NaHCO_3$ dialysis bag. The in vitro results of this revised procedure matched well with in vivo results of Schoof et al. (1995) who had fed seven Pb contaminated soils to rats. Oliver et al. (1999) used the *in vitro* extraction of Ruby et al. (1996) also reported a strong relationship ($r^2 = 0.82$) at pH 3.0 (different from pH 2.5 in the original method) to estimate bioavailability of Pb from household dust samples from the Port Pirie region, South Australia.

In another study where Superfund site soils (14,200 and 3870 mg Pg/kg) were fed to swine, Casteel et al. (1997) found that the bioavailability of Pb was 56–86% and 58–74% for the higher and lower Pb soils compared to Pb-acetate in the diet. These values are high compared to other studies, likely due to the high proportion of Pb present as cerrusite and the high proportion of Pb occurring as small particles. Kelley et al. (2002), based on work of Rodriguez et al. (1999) and Ruby et al. (1999) presented a further revision of the PBET method by eliminating the simulated intestine phase. In another test, Hettiarachchi et al. (2003) found that with regard to Pb uptake, the method proposed by Ruby et al. (1996) was 90% successful for liver and bones as the target organs (r^2 values of 0.92 or 0.88, respectively), but lower for blood and kidney. In this study, rats were either fed untreated or treated (with phosphates at two levels and MnO_2 + phosphates). Some argue that chronic accumulation in bone gives much more reliable measurements than Pb in blood or liver, causing these apparent disagreements (Pb reaches a steady state in blood, but accumulates over time in bone).

Weis and LaVelle (1991) argued against the use of rats compared to pigs, but did not report a direct comparison as was conducted by Ryan et al. (2004). Very recently Casteel et al. (2006) estimated relative bioavailability (RBA) of Pb in 19 soil and soil like materials from Superfund sites using young swine as test animals and concluded that 8 samples had RBA values within 20% of U.S.-EPA (1994) default (for RBA of Pb in soil, i.e., 60%), 6 had RBA values <40%, and 3 had >80%.

Bannon et al. (2009) following protocols of Casteel et al. (2006), Drexler and Brattin (2007), and Weis et al. (1995) conducted bioaccessibility and bioavailability tests for soil samples collected from several small arms ranges finding that the mean relative bioavailability and bioaccessibility of lead for the eight soils was about 100% (108 ± 18% and 95 ± 6%, respectively) showing such sites as a matter of serious environmental concern. Morman et al. (2009) sampled 20 uncontaminated 'background' soils along north-south and east-west transects across USA and Canada to evaluate the bioaccessibility of some trace metals. The authors, following the Drexler and Brattin (2007) procedure, concluded that "results for the <2-mm size fraction were not substantially different from those for the <250-μm size fraction for bioaccessible As, Cd, Cr, Ni and Pb (most studies of bioaccessible soil elements has focused on the fine fraction (<250 μm) because that is the size of particles which adhere to children's hands). Bioaccessibility for Pb ranged from 8.5% to 76.5% (median 23%) for the <2-mm fraction and from 3.7% to 44.8% (median 18%) for the <250-μm fraction, in this study." Cui and Chen (2010) following modified PBET procedure (Rodriguez et al., 1999) reported that bioaccessible lead for 17 soils collected from five Chinese provinces ranged from 24.6–82.5% and 2.3–57.5% in gastric and small intestinal phase phases, respectively.

In Europe, after initiation of *in vitro* methods by Ruby et al. (1992, 1993), several models/methods mimicking the human digestive tract have been developed (Molly et al., 1993; Rotard et al., 1995; Minekus et al., 1995; Hack and Selenka, 1996; Jin et al., 1999; Oomen et al., 2002, 2003a,b). Most of the models are "static models": the modified Physiologically Based Extraction Test (PBET) method conducted by the British Geological Survey, UK (Ruby et al.,

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1996); the DIN method, applied by the Ruhr-University Bochum, Germany (Oomen et al., 2002; Rotard et al., 1995), the RIVM method, *in vitro* digestion model, National Institute of Public Health and the Environment, The Netherlands (Oomen et al., 2002; Rotard et al., 1995); and the Simulator of Human Intestinal Microbial Ecology (SHIME) procedure used by LabMET, Ghent University, Belgium (Oomen et al., 2002). The only dynamic GI model reported is the TNO gastro-Intestinal Model (TIM) from the Netherlands (Minekus et al., 1995; Oomen et al., 2002). Details of each model can be found in the papers of van de Wiele (2007), and Intawongse and Dean (2006). Hund-Rinke and Kördel (2003) argued that the above mentioned models vary widely in type and concentration of the gastric juices used, digestive simulation time, and other methodologies for measurement of contaminants at different digestive phases. Oomen et al. (2002) in a comparative analysis of five bioaccessibility methods concluded that pH is probably the one single factor that has the most influence on the final soluble Pb result. A wide range of bioaccessibility values for Pb (4–91%, 1–56%, and 3–90%) were found for the three tested soils (Oomen et al., 2002) (no animal results were obtained for the soils used in evaluating bioaccessibility methods). The authors concluded that although the experimental designs of the different digestion systems are distinct, the main differences in test results of bioaccessibility can be explained on the basis of the applied gastric pH. High Pb bioaccessibility values are typically observed for a simple gastric method, which measures bioaccessibility in the gastric compartment at low pH of 1.5. Other methods that also apply a low gastric pH, and include intestinal conditions, produce lower bioaccessibility values. The lowest bioaccessibility values are observed for a gastrointestinal method which employs a higher gastric pH of 4.0. van de Wiele et al. (2007) using simulated fasted and fed conditions, in an inter-laboratory study of *in vitro* models assessing bioaccessibility of soil-bound lead (collected from Bunker Hill site and used in the human feeding test of Maddaloni et al., 1998) found that "bioaccessible lead fraction was significantly ($P < 0.05$) different between the *in vitro* methods and ranged for the fasted models from 2% to 33% and for the fed models from 7% to 29%. The *in vivo* human bioavailability data from Maddaloni et al. (1998) were $26.2 \pm 8.1\%$ for fasted conditions compared to $2.5 \pm 1.7\%$ for fed conditions. Under fed conditions, all models returned higher bioaccessibility values than the *in vivo* bioavailability; whereas three models returned a lower bioaccessibility than bioavailability under fasted conditions." The authors concluded that more optimization of *in vitro* digestion models is needed for use in risk assessment. In another study Marschner et al. (2006) failed to find any correlation between *in vitro* and *in vivo* (pigs as test animal) trials for absolute and relative bioavailability of Pb. However, Pb in the third fraction of the sequential extraction (reducible Mn oxide fraction) highly correlated with relative bioavailability based on kidney, liver and total Pb uptake. Bioaccessible Pb ranged from 11–56% with use of powdered milk and 3–20 in its absence for *in vitro* system used in this study. Roussel et al. (2010) evaluate bioaccessibility of heavy metals from urban gardens using the unified BARGE method (UBM) and reported 62% Pb bioaccessibility in gastric phase and 32% in intestinal phase. In another study employing an in vitro gastrointestinal test, bioaccessibility of Pb in the soil samples were 39.1% and 6.9% in the stomach phase and intestinal phase, respectively and exposure risk of Pb in the soils ranked in the order of: industrial area/urban parks > residential area/road side (Lu et al., 2011).

There is a need to realize that specific mineral phase and the degree of crystallinity associated with soil Pb are also key factors in Pb bioavailability (Hall and Tinklenberg, 2003; Yang et al., 2003). Yet to reverse this trend, much work is required to establish baseline bioavailability measurements and to develop complementary methods that are capable of predicting bioavailability across a wide range of impacted media in a cost-efficient manner. Great effort has been made in conducting research to develop bioaccessibility methods which try to match all mammalian digestion processes without a valid bioavailability measurement for evaluation of the reliability of the bioaccessibility extraction. To the authors, the most important criterion for evaluation of an *in vitro* bioaccessibility method is the correlation with an appropriate *in vivo* bioavailability model. Actually, the simpler and less expensive the bioaccessibility method can be made, the better, as long as the correlation with bioavailability is high. Further, it is necessary for the tests to be reproducible in laboratories across the globe, which has not been the case for many of the complex bioaccessibility methods in the literature. Further, for such methods to be relevant to testing of remediation methods, changes in bioavailability due to field treatments should be reflected in the bioaccessibility test results (Ryan et al., 2004).

As noted above, the Drexler and Brattin (2007) Relative Bioavailability Leaching Procedure (RBALP) uses the simplified stomach phase only. They had access to the diverse soil materials from Superfund sites which had been fed to juvenile swine using the Casteel et al. (2006) procedure. The RBALP test uses 0.4 M glycine·HCl with enough HCl to buffer the solution at pH 1.5 to mimic fasting stomach pH; the extraction is conducted at 37 °C for 1 h, using 1 g dry soil or dust per 100 mL extraction fluid. They indicate that if the pH of the extraction fluid is raised during the test that the pH should be manually re-adjusted to 1.5 until it stays at that pH for the extraction period. The correlation with swine RBA results was found to be $r^2 = 0.82$ at pH 1.5, while the correlation was $r^2 = 0.75$ when conducted at pH 2.5. But as noted above, conducting the extraction at pH 1.5 left it insensitive to the highly effective soil remediation treatments using phosphate tested at Joplin, which were shown to lower Pb bioavailability by feeding to swine, rats and humans (Ryan et al., 2004). Thus we believe this method should be conducted at pH 2.5 so that the results have relevance to soil remediation (similar effects of extractant pH upon significant variations in bioaccessible Pb, with and without phosphate amendments, have also been reported by Moseley et al. (2008). Further, the RBALP extraction test method at pH 1.5 gave a much smaller reduction in soil Pb bioavailability upon phosphate treatment (18%) than found with human volunteers fed the Joplin soil (1% H_3PO_4 treated and sampled after 18-months) on fasting (69%) and lower reduction than measured by the swine and rat feeding tests. This is clear evidence that more soil Pb remediation test materials should be fed to humans to provide the definition of remediation of risk that seems to be obtainable with inexpensive technologies (Ryan et al., 2004). Another attempt to validate an extraction procedure was reported by Schroder et al. (2004) who fed 18 soils to swine following the Casteel et al. (2006) procedure, and tested stomach and intestinal phases on an "*in vitro* Gastrointestinal" (IVG) extraction method. The presence of the dough used to dose the test soil to swine significantly reduced IVG-Pb, perhaps due to the presence of Ca, phosphate or phytate. Their method used pH 1.8, and added porcine enzymes. Strong correlation was obtained between IVG and RBA results.

Although the present *in vitro* bioaccessibility measurement methods are much lower cost than an animal feeding study, we believe that the current *in vitro* methods could be made simpler and much less expensive for application to determine the risk of urban soils for gardening. With the availability of soils which have been fed to test animals as part of the Joplin study, these authors evaluated different ways to simplify the bioaccessibility extraction, including effect of pH, temperature, shaking time, soil to solution ratio, shaking speed, and other variables. The focus was inexpensive measurement of bioaccessible soil Pb for urban garden soils to

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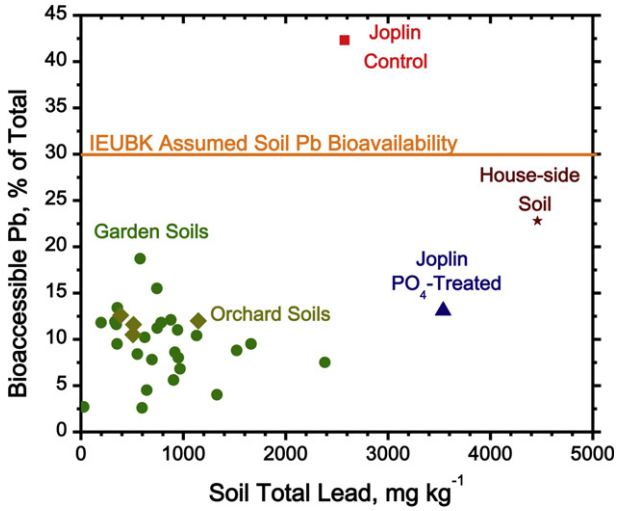

Fig. 1. Bioaccessibility of Pb in soils from the Joplin field test of phosphate remediation treatments, and from urban garden and orchard soils (Zia et al., submitted for publication).

support public decisions to encourage gardening by US citizens. Because inner city soils are nearly all Pb contaminated to a significant extent (Mielke et al., 1983), citizens should test their soils before install gardening on urban soils. And because soil surrounding older painted houses can be highly Pb contaminated, or housing may have been installed on soils which had been used for orchards sprayed with Pb-arsenate, this recommendation to obtain soil analysis before undertaking gardening applies to rural as well as urban gardens. The newly developed method of Zia et al. (submitted for publication) for soil Pb bioaccessibility is less than one-tenth as expensive as current methods and has revealed that urban soils have only 5–10% bioaccessible Pb of total Pb level (Fig. 1).

3. Conclusions

The bioavailability of Pb in many urban soils has declined due to normal chemical and physical reactions in the environment over time. Vegetable garden soils in particular receive phosphate and compost amendments which hasten the formation of lesser-bioavailable forms of Pb in the soil. Although Pb in Superfund site soils may remain highly bioavailable, this review indicates that the fractional bioavailability of Pb in the IEUBK model should be adjusted for urban soils in general, and that testing of urban soil Pb bioaccessibility should be recommended rather than total soil Pb (the basis for present advice from EPA and HUD). The Agricultural Extension Service recommendations regarding safety of urban gardening should take into account the increasing evidence that many urban soils have low fractional bioavailability of soil Pb and comprise far lower risk than previously presumed. On the other hand, because of the Pb contamination of human-affected soils, citizens should obtain soil Pb bioaccessibility tests for soils to which their children will be exposed or where they seek to garden.

4. Further work needed

Ultimately, all bioaccessibility tests have to be validated by correlation with animal bioavailability tests. With the evidence that soil Pb bioavailability to swine is not necessarily well correlated with bioavailability to humans (Ryan et al., 2004), and that human feeding tests can be inexpensive and safe to conduct (Maddaloni et al., 1998), it is apparent that a number of soils need to be fed to humans and made available to developers of bioaccessibility tests. The soils should include untreated soils and those soils treated with methods which have been shown to significantly reduce soil Pb bioavailability in previous tests (e.g., phosphate and Fe/Mn oxides). Large volumes of the soils should be collected and homogenized to allow subsequent use by others who will test the validity of proposed bioaccessibility tests.

Disclosure statement

All the authors of this manuscript declare that they have no conflicting interests. Further, none of the co-authors have any competing interests for their environmental lead research.

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